Effects of Soil Insects on Populations and Germination of Fungal Propagules

E. A. CURL and R. T. GUDAUSKAS, Department of Botany, Plant Pathology, and Microbiology; J. D. HARPER, Department of Zoology-Entomology; and C. M. PETERSON, Department of Botany, Plant Pathology, and Microbiology, Auburn University, Auburn, AL 36849, U.S.A.

Interactions between components of the soil microflora and the microfauna (excluding nematodes) have been largely neglected in studies relating to the ecology of soilborne plant pathogens. The microarthropods, comprised principally of the Collembola (springtails) and the Acarina (mites), are the most abundant of the soil-inhabiting animals exclusive of nematodes and protozoa (7).

The order Collembola consists of minute animals, usually 1–3 mm long, in the subclass Apterygota. The collembolan suborder Arthropleona is well represented in soil below the surface layer and, like the microflora, is generally more abundant in close proximity to roots (11). Many species are predominantly mycophagous (9). Wiggins and Curl (10) suggested that the feeding activities of these insects might affect the competitive advantage of a pathogen at the root surface and influence disease incidence or severity. Subsequently, a suppressive effect of mycophagous Collembola on *Rhizoctonia solani* Kuehn and cotton-seedling disease was reported (5). An overview of the potential role of mycophagous insects in rhizosphere ecology relating to root disease has been published (6).

The present investigation was designed to obtain more specific information on the destructive feeding capacity of two collembolan species in relation to inoculum density of selected pathogenic fungi and the biological control agent *Trichoderma harzianum* Rifai.

MATERIALS AND METHODS

The collembolan species, *Proisotoma minuta* Tullberg (Isotomidae) (Fig. 1) and *Onychiurus encarpatus* Denis (Poduridae), used in these studies were extracted from soil close to the rhizosphere of various crop plants by the modified Tullgren extraction system described by Wiggins and Curl (10). The insects were further multiplied and maintained in deep petri dishes on a substrate of plaster of Paris and charcoal (9:1) with bakers' yeast as a food source. The two species were always used in mixtures, with *P. minuta* comprising about 90% of the population.

Effect on mycelia. An assessment was made of the capacity of the insects to restrict mycelial growth of six soil fungi on the surface of Czapek's agar within a 24-hr period. Mycelial disks (7 mm in diameter) from young potato-dextrose agar (PDA) cultures of *Rhizoctonia solani*, *Fusarium oxysporum* Schlect. f. sp. *vasinfectum* (Atk.) Snyder and Hansen, *Sclerotium rolfsii* Sacc., *Macrophomina phaseolina* (Tassi) Goid., *Verticillium dahliae* Kleb., and *Trichoderma harzianum* were applied to the agar surface 12 mm from the petri dish edge. In dishes that were to receive insects, a disk of sterile PDA was also placed near the opposite edge. Approximately 200 insects per dish were added immediately to these dishes; other dishes were not infested. These cultures were prepared in triplicate.

After 24 hr, mycelial extension in the presence and absence of insects and the insect distribution pattern or "attitude" in relation to food source were determined by microscopic examination.

Insect attraction to and effect on spores. Before soil studies, tests were conducted to observe spore-insect relationships on a water-agar surface. Chlamydospores of *F. oxysporum* f. sp. *vasinfectum* were produced in a soil extract liquid medium and separated from the mycelium by sonification (sonicator cell disrupter, model W. 185F, Heat Systems Ultrasonic, Inc.) for 2 min at power control setting 4, followed by ultrafine screening. Conidia of *T. harzianum* were obtained by flooding PDA cultures with sterile water. Spores were washed on 0.45-μm Millipore filters and concentrations adjusted by hemacytometer counts to about 1×10^5 spores per milliliter for *Fusarium* and 2×10^6/ml for *Trichoderma*.

Four 13-mm-diameter circles were drawn equidistant on the bottoms of 15 petri dishes containing water agar. A single drop of spore suspension was placed within two of the circles on the agar surface of 10 dishes, and a drop of spore-wash water was placed within the other two circles. Approximately 150 Collembola were placed in the center of each of these dishes. For the remaining five dishes, all four circles received a spore drop but no insects were added. After 12 hr at 27° C, the number of Collembola per circle, the number of spores per microscopic field, and the percentage of germination of spores were recorded.

Spores in soil. Suspensions of *F. oxysporum* f. sp. *vasinfectum* chlamydospores and *T. harzianum* conidia were prepared as described above. One milliliter was applied to 25-mm Millipore membrane filters mounted over vacuum, leaving about 10^5 *Fusarium* spores or 10^6 *Trichoderma* spores on each filter. These filters were buried in either sterilized or nonsterilized sandy loam (80 g) adjusted to 50–60% moisture-holding capacity and contained in 100-ml jars with loosely applied screw caps. The soil used was collected from a fertile, experimental cotton (*Gossypium hirsutum* L.) field at Auburn, Alabama, and air-dried to exclude natural insect populations. The cropping history of the field has been described (11). Insects were introduced into some jars at approximately 200/jar (1,600/kg of soil); other jars of soil were kept without insects. After 10 hr at 27° C, the filters were gently removed and processed for microscopic examination according to the original method of Adams (1). Data recorded for *Fusarium* consisted of the number of chlamydospores in two microscopic fields on each of four filter disks per treatment and the percentage of germination of spores. For *Trichoderma*, data were taken from three microscopic fields on three filter disks. Following the experiment, insects were extracted from the soil to verify survival.

Sclerotia. Laboratory tests were performed to assess the direct effects of Collembola on sclerotia of three pathogenic fungi. Mature sclerotia of *S. rolfsii* were produced on PDA cultures, and microsclerotia of *V. dahliae* and *M. phaseolina* were obtained from 3-week-old cultures on Czapek's agar. Microsclerotia were separated from the agar and mycelia by chopping in a microblender with 35 ml of sterile water for 2 min followed by passage through a fine-mesh sieve and resuspension in water. The sclerotium concentration was adjusted to about 100 microsclerotia per milliliter.

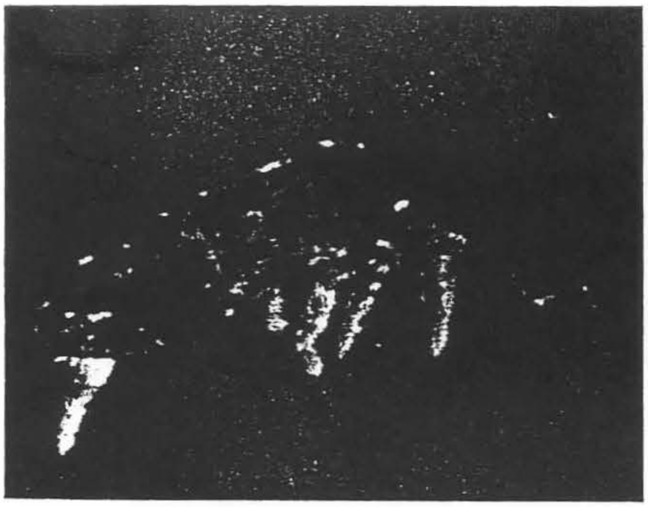

Fig. 1. *Proisotoma minuta*, a soil-dwelling, mycophagous insect of the order Collembola (×100).

Twenty sclerotia of *S. rolfsii* were placed in each of 12 petri dishes on the packed and smoothed surface of nonsterilized sandy loam (10% moisture) taken from a fertile cotton field. Approximately 200 Collembola were added to six of the dishes, and six were left noninfested. For the microsclerotial fungi, one drop of sclerotial suspension was applied to the surface of 2% water agar 15 mm from the dish edge, and a drop of sterile water was applied near the opposite edge. Collembola were added to the centers of five dishes, and five remained noninfested. After 16 hr of incubation in the dark at 27° C, the numbers of sclerotia and percentage of germination were recorded.

Finally, a test was conducted with microsclerotia of *M. phaseolina* in which 1 ml of a sclerotium suspension (100/ml) was applied to Millipore filters and buried in jars of nonsterilized soil in the manner described for spores. Collembola (200/jar) were added or not added and, after 24 hr, filters were recovered and the percentage of germination was recorded for sclerotia within five stereoscopic microscope fields for five disks of each treatment.

RESULTS

Effect on mycelia. Comparisons of infested and noninfested cultures clearly demonstrated the capacity of Collembola to suppress colony growth of certain fungi (Fig. 2). The distribution pattern of insects on the agar surface indicated their strong attraction to the fungal food source, although frequently no hyphal extension was visible to the unaided eye (Fig. 3). The insects were not attracted to sterile PDA disks. Apparently, visible hyphae on *R. solani* disks were consumed so rapidly that the insects dispersed widely in search of other food sources. In contrast, it is evident that the mycelia of *S. rolfsii* and *T. harzianum* repelled insects. However, insect activity accounted for a 75% reduction in germination of *S. rolfsii* sclerotia on the surface of nonsterilized soil and reductions of 65 and 50% for microsclerotia of *V. dahliae* and *M. phaseolina*, respectively, on an agar surface.

Results of a single preliminary test with microsclerotia of *M. phaseolina* on buried Millipore filters in nonsterilized soil showed very low germination overall,

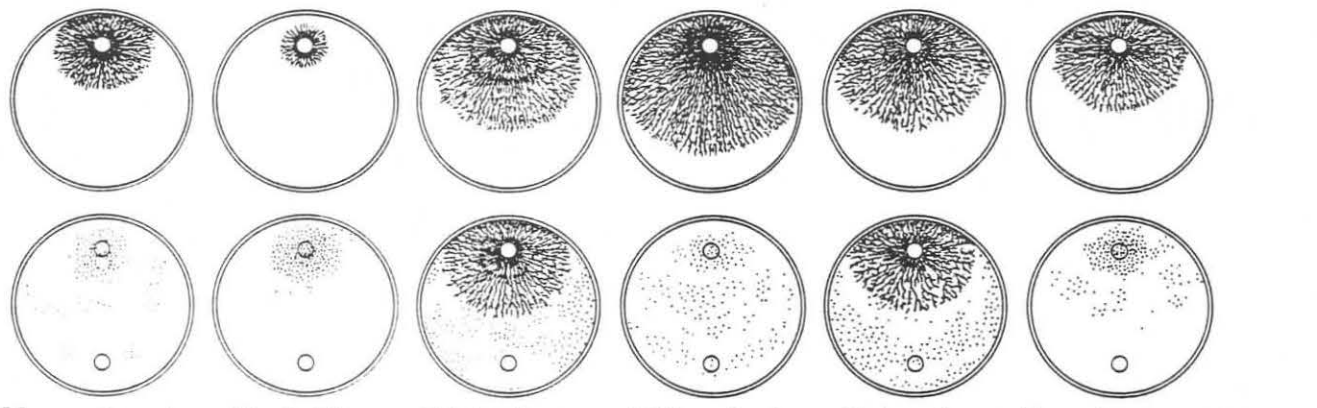

Fig. 2. Suppression of fungal growth (bottom) by Collembola on Czapek's agar, compared with colony growth in the absence of insects (top).

but they also showed a significant reduction when insects were present. The mean germination in nonsterilized soil without insects was 25.7%, compared with 14.3% in nonsterilized soil + insects. This appeared to result from grazing on the sclerotial germ tubes, preventing their normal development.

DISCUSSION

Most species of Collembola are regarded as saprophagous and microphagous, some being more specifically mycophagous and rarely known to injure plant roots. Their distribution and feeding activities are largely related to the availability of the microflora (4); therefore, populations are often larger in the root zone than distant from roots (11). That these very active insects can consume relatively large amounts of fungal material is evident from reports of mycelia and spores found in the gut contents (2,3,8). They have not, however, been given proper consideration as interacting components of the rhizosphere where activities of root-infecting fungi may be influenced.

The present study has established that two representative species of the soil-dwelling Collembola are attracted to mycelia and spores as food sources and can cause significant reductions in inoculum density. Although the mycophagous Collembola are generally believed to be unspecialized feeders, having no preferences for different fungi, their partial aversion to mycelia of *S. rolfsii* and *T. harzianum* indicates that exceptions occur.

The Collembola tested can reduce spore populations even in nonsterilized soil where other food sources are available (Table 1). Many ingested spores of *F. oxysporum* f. sp. *vasinfectum* and *T. harzianum* passed

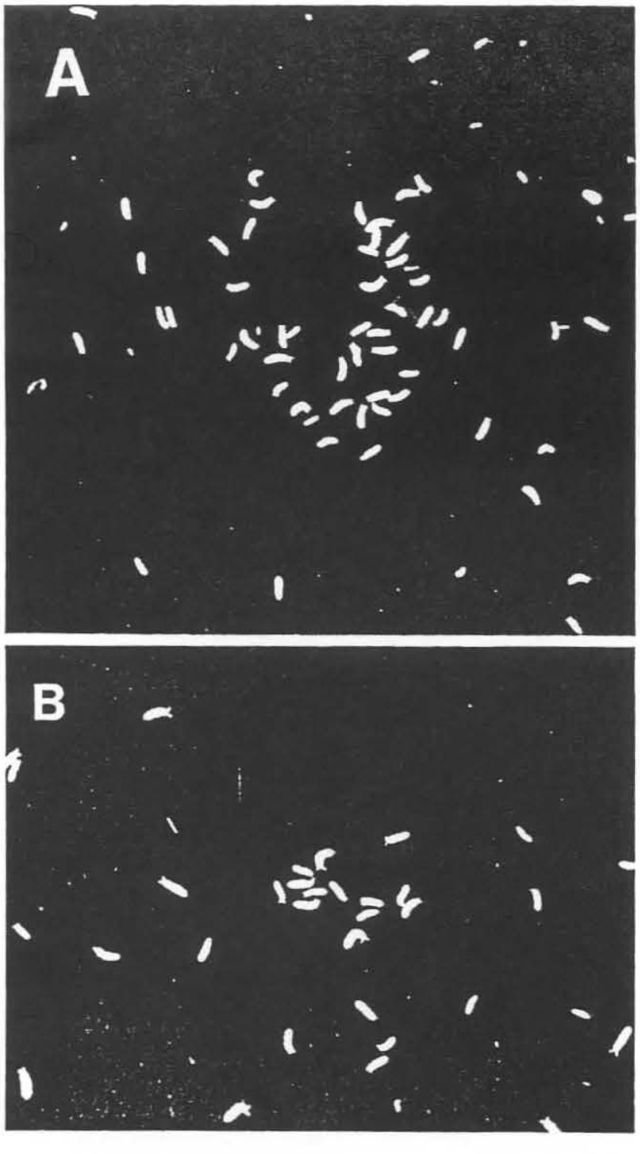

Fig. 3. Relation of Collembola to fungal spores on a water agar surface. (A) Attraction of insects to a drop of washed chlamydospores of *Fusarium oxysporum* f. sp. *vasinfectum* and (B) conidia of *Trichoderma harzianum*.

Table 1. Effects of mycophagous Collembola on fungal spores in sterilized and nonsterilized soil

	Fusarium chlamydospores		*Trichoderma* conidia	
Treatment	Number of spores[w]	Germination[x] (%)	Number of spores[y]	Germination[x] (%)
		Sterile soil		
Without insects	171.6 a[z]	74.6 a	120.9 a	35.5 a
With insects	76.4 b	47.2 b	20.8 b	29.6 a
		Nonsterile soil		
Without insects	195.5 a	30.6 a	194.2 a	31.4 a
With insects	16.1 b	21.6 a	47.6 b	33.6 a

[w] Mean number of spores counted in two fields on each of four Millipore filter disks.

[x] Germination represents both noningested spores and spores ingested by insects and redeposited in fecal pellets on the filter disks.

[y] Mean number of spores counted in three fields on each of three Millipore filter disks.

[z] Pairs of means followed by the same letter do not differ from each other at the 0.01 level of probability according to the Student *t* test.

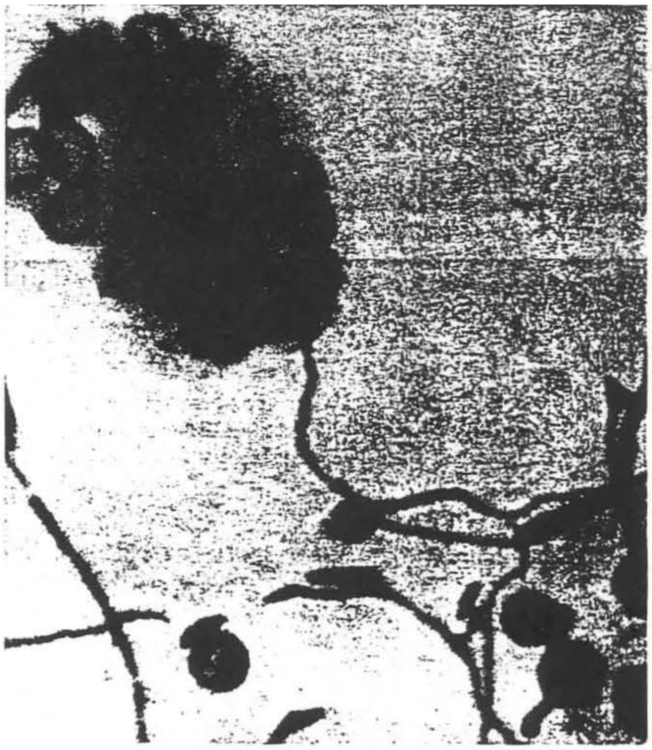

Fig. 4. Insect fecal pellet containing chlamydospores of *Fusarium oxysporum* f. sp. *vasinfectum* (×600).

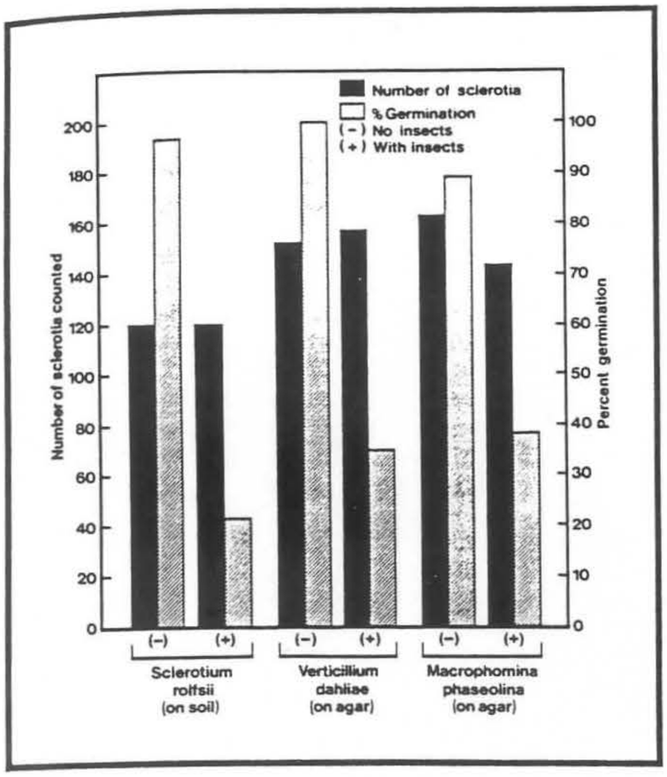

Fig. 5. Effects of Collembola on the sclerotia of three plant pathogenic fungi.

through the animal gut and were redeposited intact in fecal pellets (Fig. 4). The percentage of viability of these spores was not determined in this study; however, the combined germinability of noningested chlamydospores and those released from disrupted fecal pellets was reduced in sterilized soil.

The Collembola in this study apparently cannot readily destroy sclerotia; rather, they consume the hyphae of germinating sclerotia so efficiently that, presumably, they might render the propagules ineffective as viable inocula (Fig. 5). It is not understood why these insects feed on very young hyphae emerging from sclerotia of *S. rolfsii* and ingest conidia of *T. harzianum*, yet do not favor the mycelia of these fungi as food sources.

It seems evident that soil microarthropods, especially the Collembola, when present in sufficient numbers, may play an important role in soil-fungus ecology, conceivably altering the inoculum density and potential of plant pathogens and affecting the activities of natural biological control agents such as *Trichoderma* spp. Since these animals are attracted to fungal food sources of high density, their impact upon the quantitative and qualitative nature of the rhizosphere flora should be investigated further.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. Adams, P. B. 1967. A buried membrane filter method for studying behavior of soil fungi. Phytopathology 57:602-603.
2. Anderson, J. M., and Healey, I. N. 1972. Seasonal and interspecific variation in major components of the gut contents of some woodland Collembola. J. Anim. Ecol. 41:359-368.
3. Christen, A. A. 1975. Some fungi associated with Collembola. Rev. Ecol. Biol. Sol. 12:723-728.
4. Christiansen, K. 1964. Bionomics of Collembola. Annu. Rev. Entomol. 9:147-178.
5. Curl, E. A. 1979. Effects of mycophagous Collembola on *Rhizoctonia solani* and cotton seedling disease. Pages 253-269 in: Soil-Borne Plant Pathogens. B. Schippers and W. Gams, eds. Academic Press, London.
6. Curl, E. A. 1982. The rhizosphere: Relation to pathogen behavior and root disease. Plant Dis. 66:624-630.
7. Kevan, D. K. M. 1965. The soil fauna—its nature and biology. Pages 33-51 in: Ecology of Soil-Borne Plant Pathogens. K. F. Baker and W. C. Snyder, eds. University of California Press, Berkeley.
8. Poole, T. B. 1959. Studies on the food of Collembola in a Douglas fir plantation. Proc. Zool. Soc. Lond. 132:71-82.
9. Wallwork, J. A. 1970. Ecology of Soil Animals. McGraw-Hill, London.
10. Wiggins, E. A., and Curl, E. A. 1979. Interactions of Collembola and microflora of cotton rhizosphere. Phytopathology 69:244-249.
11. Wiggins, E. A., Curl, E. A., and Harper, J. D. 1979. Effects of soil fertility and cotton rhizosphere on populations of Collembola. Pedobiologia 19:17-82.